EPA
United States
Environmental Protection
Agency

Microphysiological Systems: A Cellular Dilemma

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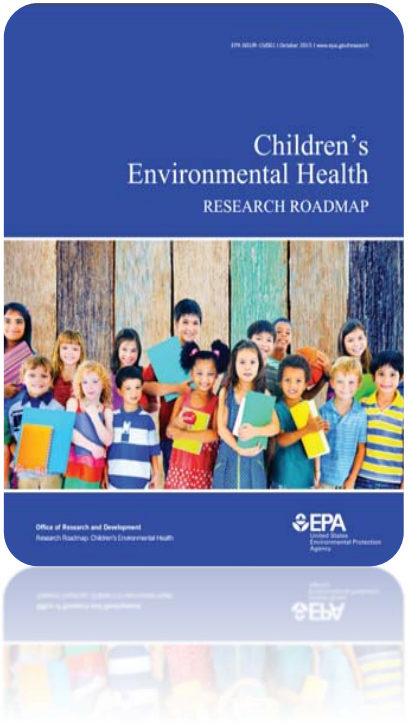
Advanced Experimental Toxicology Models Branch
Biomolecular and Computational Toxicology Division
Center for Computational Toxicology and Exposure
ORD/ US EPA

MPS: A Cellular Dilemma

Andrew Schwab – Former Post Doc
Harriette Nichols
Susan Jeffay
Tim Shafer
Kathleen Wallace
Theresa Freudenrich
Tom Knudsen

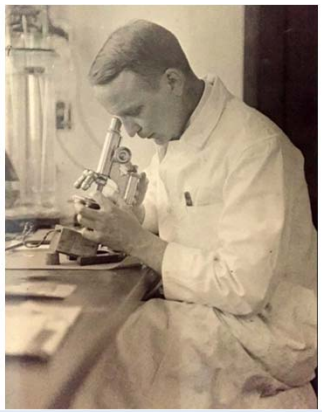


January 2021 Birth Defects Prevention Month

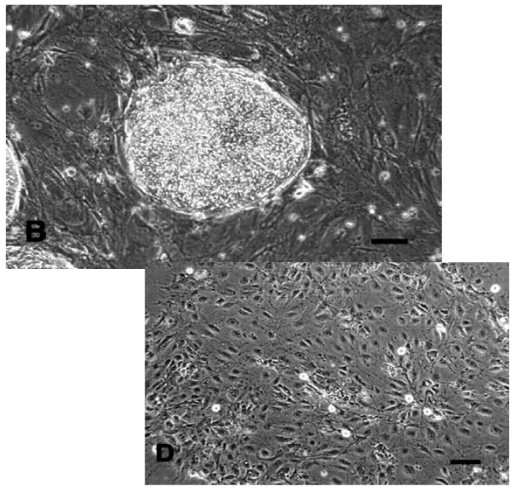
DISCLAIMER: The views expressed are those of the presenter and do not necessarily reflect Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use .

Incredible Advancements in the Tools and Approaches to Cell Culture

Wilhelm Roux

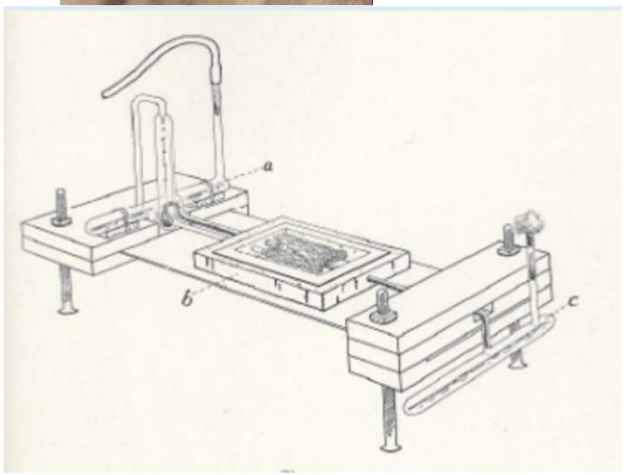


Montrose T Burrows
1912



Thompson et al., Science 06 Nov 1998: 282(5391), 1145-1147

Alexis Carrel
1923

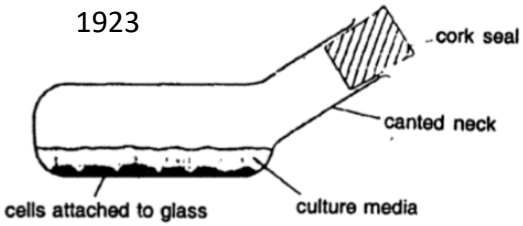


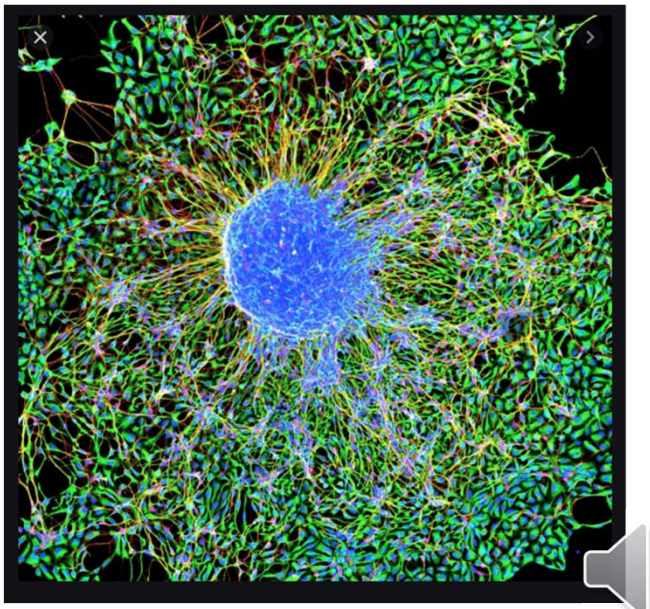
A method of furnishing a continuous supply of new medium to a tissue culture In Vitro†

Montrose T. Burrows

First published:March 1912 | https://doi.org/10.1002/ar.1090060307 | Citations: 21

† Read before the American Association of Anatomists, December 27, 1911, at Princeton, N. J.

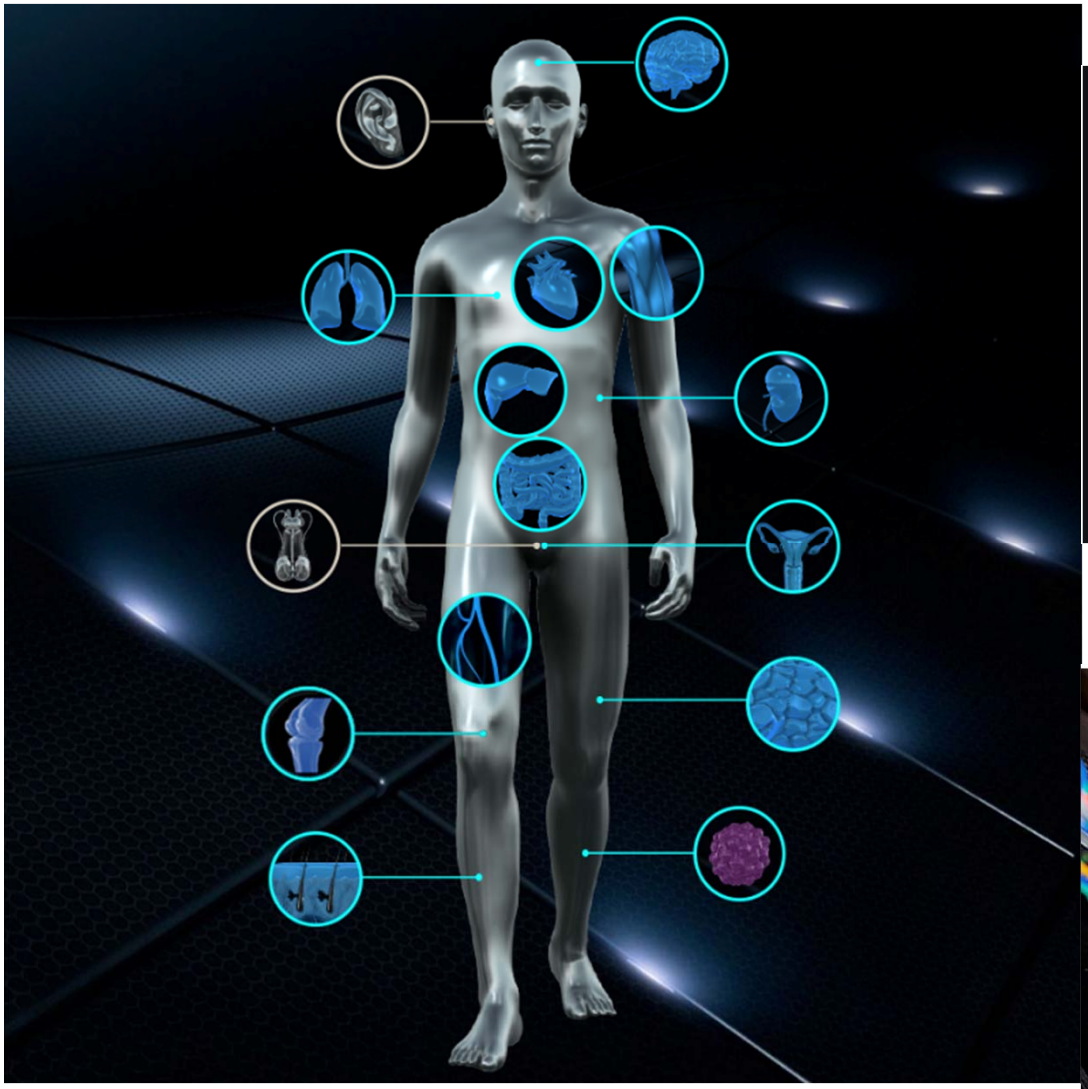




https://www.the-scientist.com/image-of-the-day/image-of-the-day-monkeying-around-34841

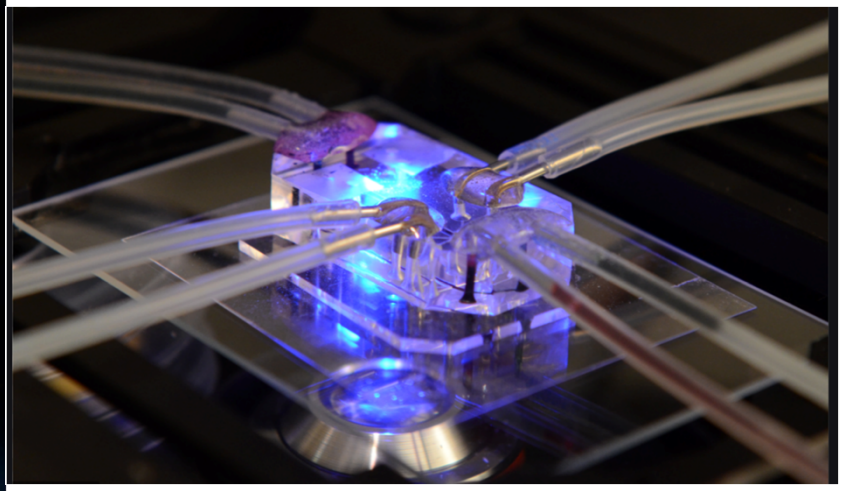
UNIVERSITY OF WISCONSIN-MADISON, S.C. VERMILYEA, S. GUTHRIE, T.G. GOLOS, M.E. EMBORG

Organ-on-Chip, Microphsyiological Models are being developed for many organs

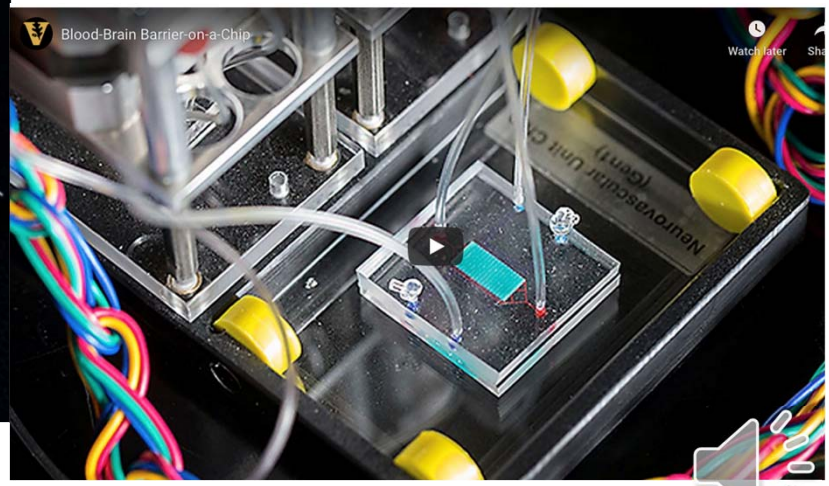


https://ncats.nih.gov/tissuechip/chip

Lung on a Chip wyss.harvard.edu



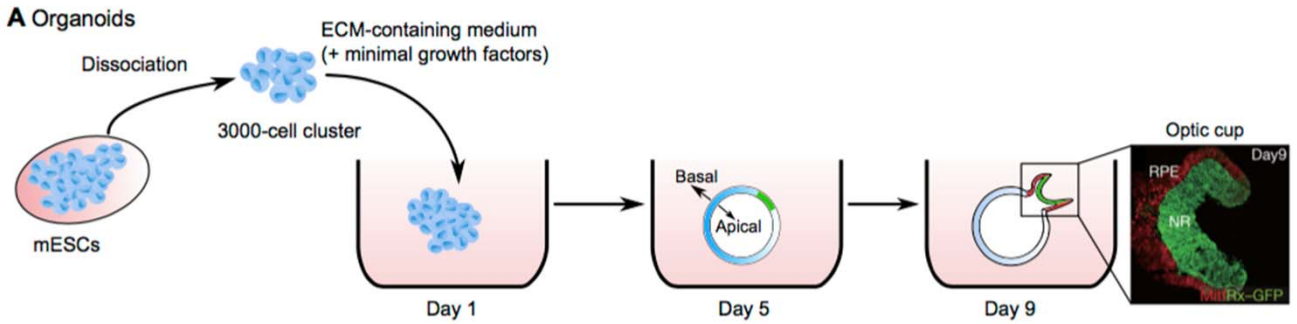
NeuroVascular Unit Chip

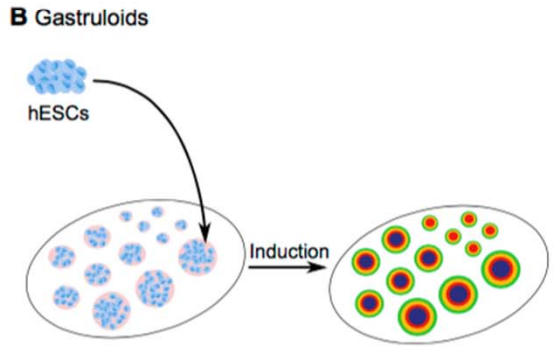


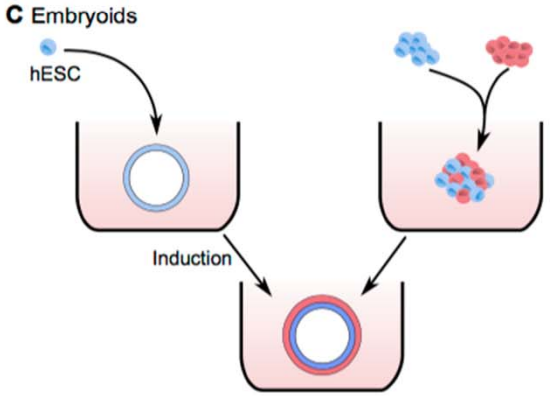
https://news.vanderbilt.edu/2016/12/06/
blood-brain-barrier-on-a-chip-sheds-new-light-on-silent-killer/

Incredible Advancements in the Tools and Approaches to Cell Culture

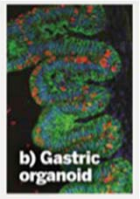
Self Organization in Organoids, Gastruloids and Embryoids

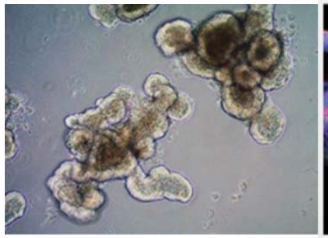


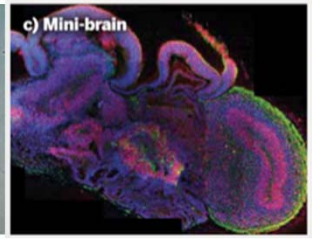


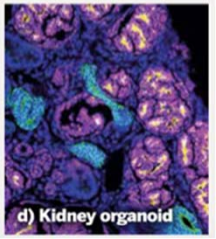


Simunovic and Brivanlou, Development (2017) 144, 976.



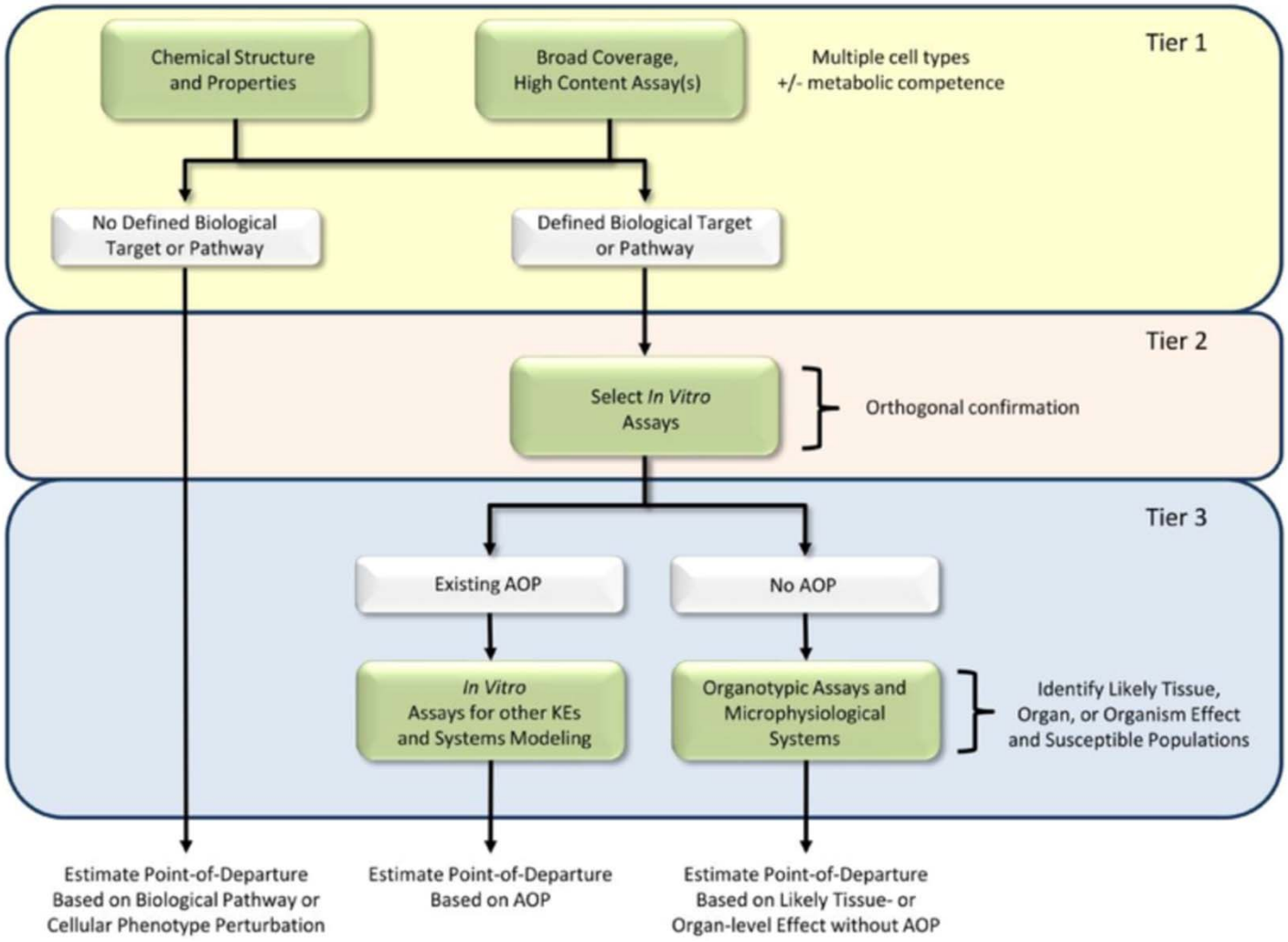






https://www.rsb.org.uk/biologist/158-biologist/features/1830-from-organoids-to-gastruloids

MPS models as part of a tiered testing approach for assessing chemical effects



TOXICOLOGICAL SCIENCES, 169(2), 2019, 317–332

doi: 10.1093/toxsci/kfz058
Advance Access Publication Date: March 5, 2019
Forum

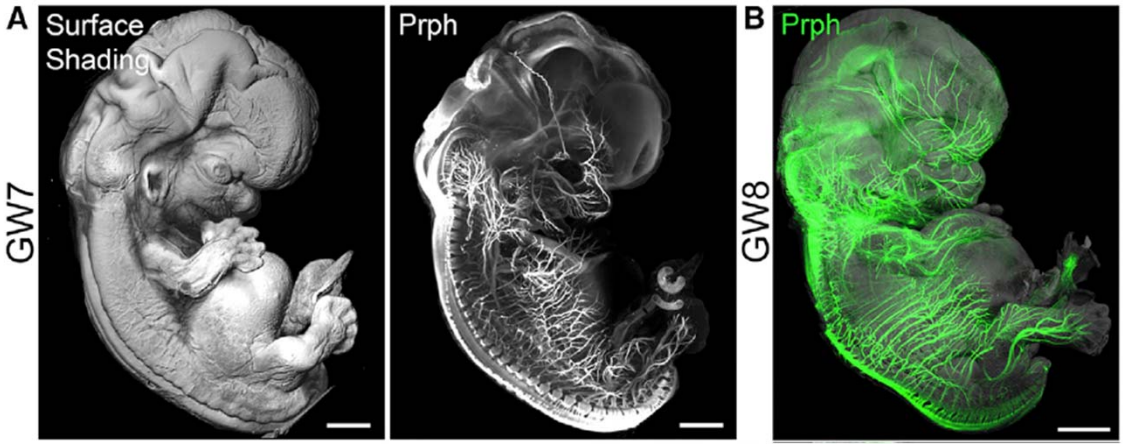
The Next Generational Blueprint for Computational Toxicology at the US EPA

Birth Defects

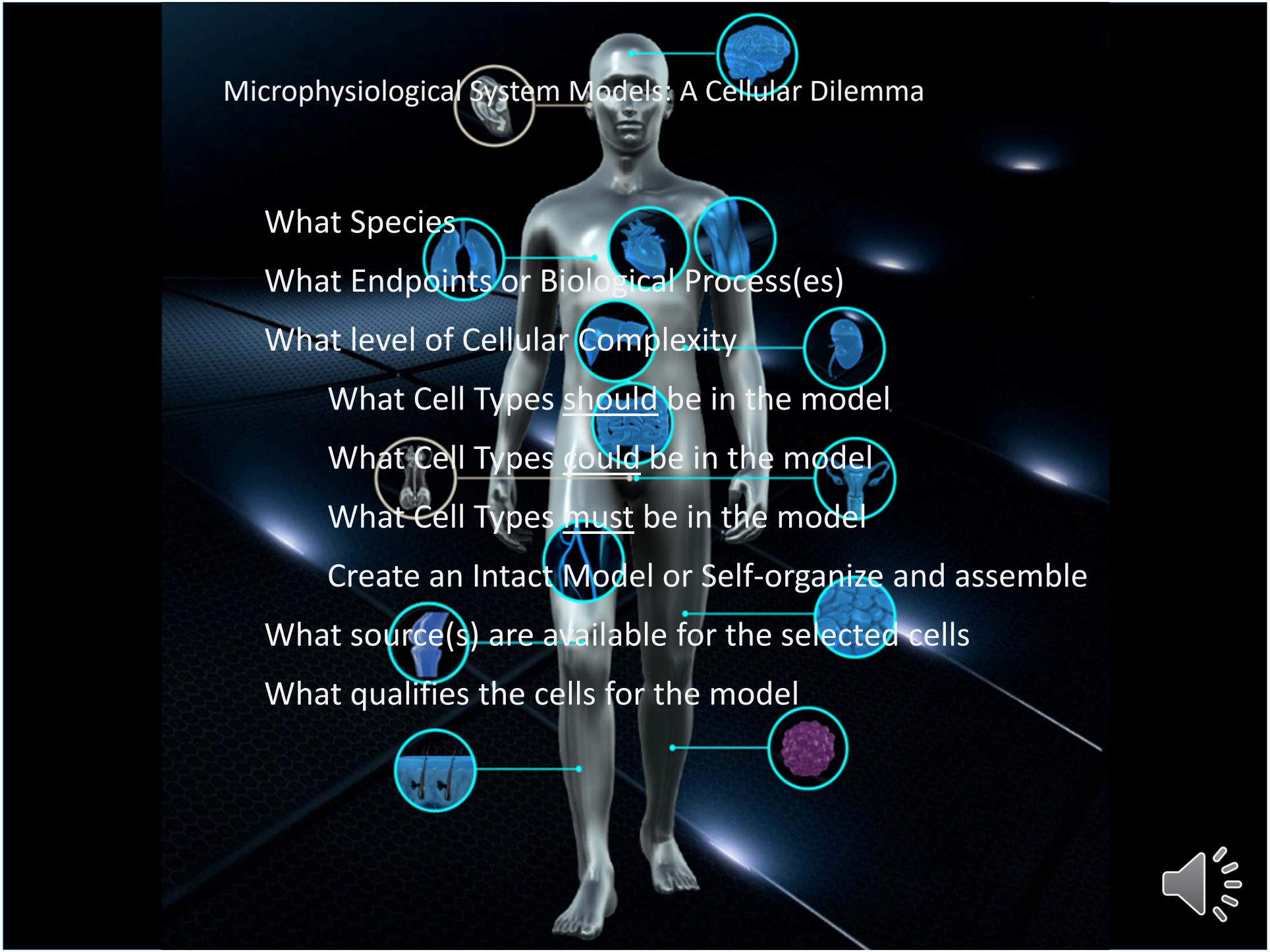
A child with a birth defects is born every 5 minutes in the US

1 child in 33 will be born with a birth defect

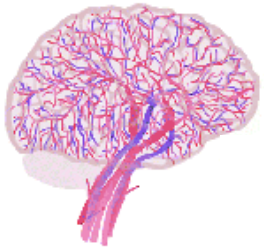
Birth Defects are the leading cause of Infant mortality in the US



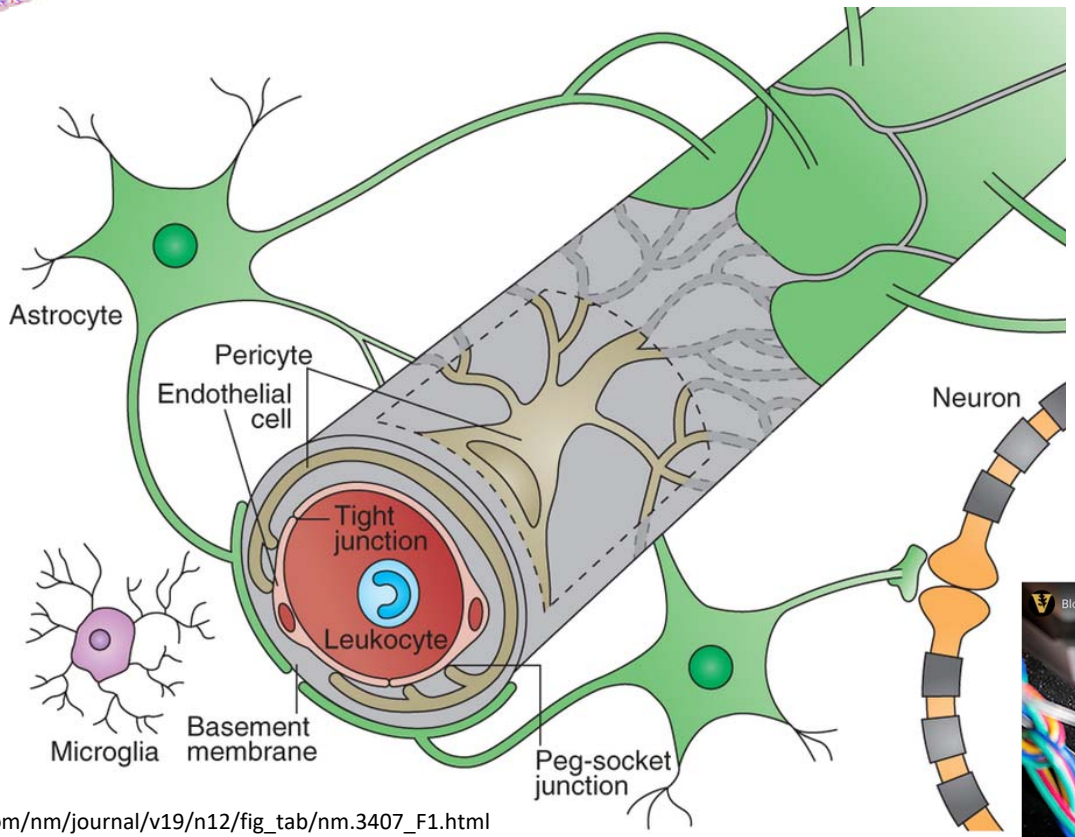
Belle et al., 2017, Cell 169, 161-173


Microphysiological System Models: A Cellular Dilemma
What Species
What Endpoints or Biological Process(es)
What level of Cellular Complexity
What Cell Types should be in the model
What Cell Types could be in the model
What Cell Types must be in the model
Create an Intact Model or Self-organize and assemble
What source(s) are available for the selected cells
What qualifies the cells for the model

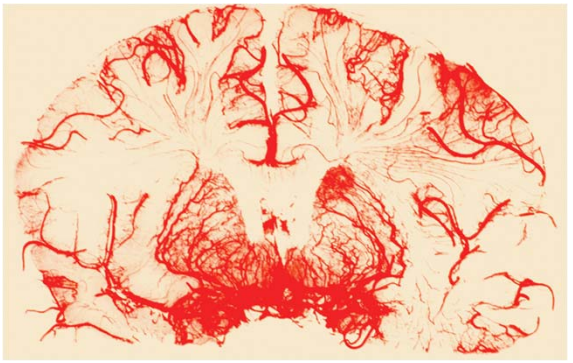
Develop a human embryo-neurovascular unit (NVU) to quantitatively assess the impact of chemical-induced disruption of neural morphogenesis.



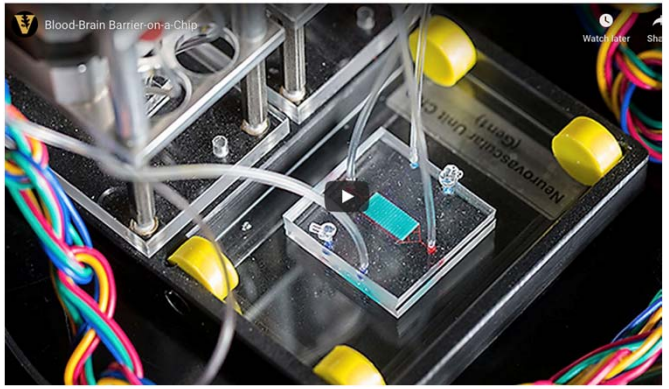
Neurovascular Unit



http://www.nature.com/nm/journal/v19/n12/fig_tab/nm.3407_F1.html



NeuroVascular Unit Chip



https://news.vanderbilt.edu/2016/12/06/

Home > Biomicrofluidics > Volume 9, Issue 5 > 10.1063/1.4934713

Open . Published Online: 26 October 2015 Accepted: October 2015

Recreating blood-brain barrier physiology and structure on chip: A novel neurovascular microfluidic bioreactor

Biomicrofluidics 9, 054124 (2015); https://doi.org/10.1063/1.4934713

Jacquelyn A. Brown[1,2], Virginia Pensabene[1], Dmitry A. Markov[1,2], Vanessa Allwardt[3], M. Diana Neely[4], Mingjian Shi[5], Clayton M. Britt[3], Orlando S. Hoilett[3], Qing Yang[3], Bryson M. Brewer[6], Philip C. Samson[1,2], Lisa J. McCawley[1,2,7], James M. May[8], Donna J. Webb[5], Deyu Li[6], Aaron B. Bowman[4], Ronald S. Reiserer[2,3], *and* John P. Wikswo[1,2,3,8]

Key Components of the embryo-NVU model

Human Cell Types:

Endothelial Cells

hBMVECs – Cell Systems

Pericytes

hBVPs - ScienCell

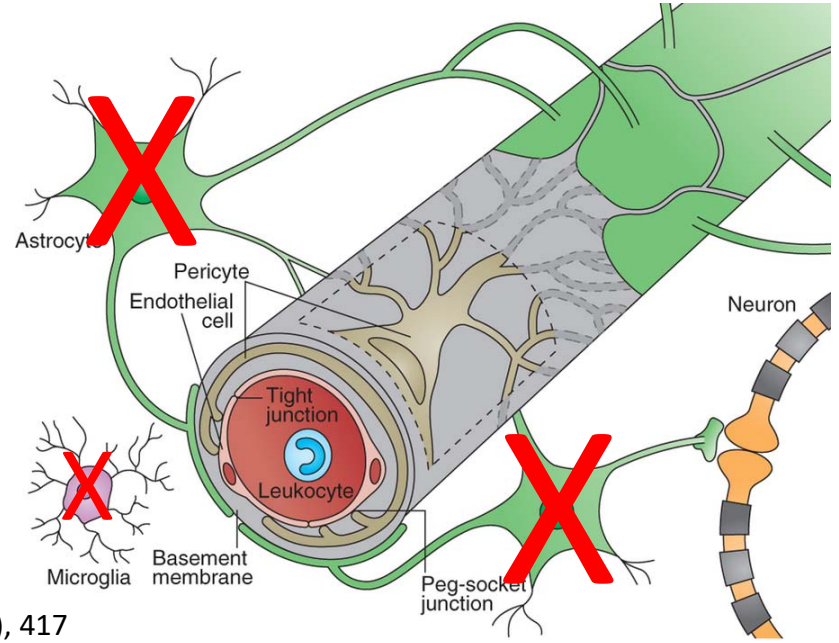
Neuroprogenitor Cells

form neurons & astrocytes

EZ spheres (floating neural progenitors)

Allison Ebert et., al., 2013. Stem Cell Research 10(3), 417

Neurovascular Unit

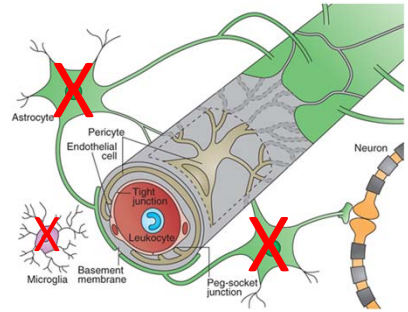


- Embryonic microglia are from Yolk Sac primitive macrophage progenitors
- Astrocytes first appear in the human cortex at around 20 weeks of gestation

Human pluripotent stem cell-based in vitro models that reflect human physiology and function have the potential to offer an effective approach for assessing chemical effects.

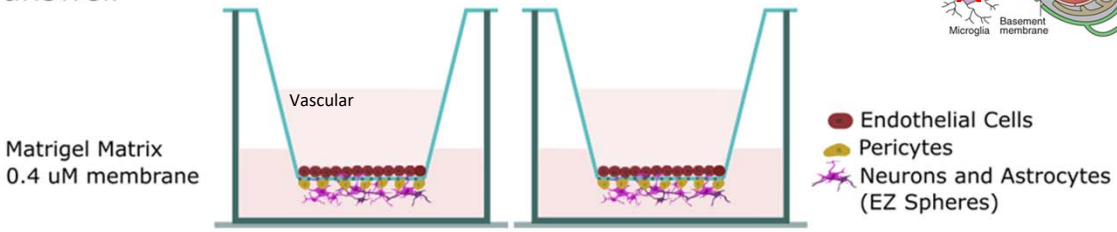
Andrew Schwab et al., - Development of complementary 3-dimensional human neurovascular unit models using static transwells and dual-compartment microfluidic devices. In Revision

Establishing in vitro Static and Dynamic models of the NVU



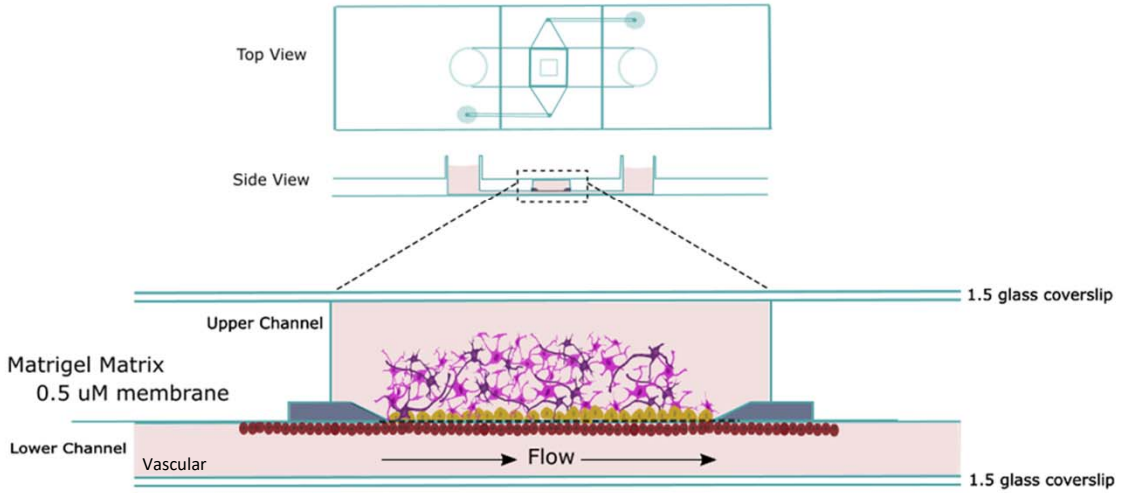
3D Static

A. Transwell



B. Ibidi Microfluidic

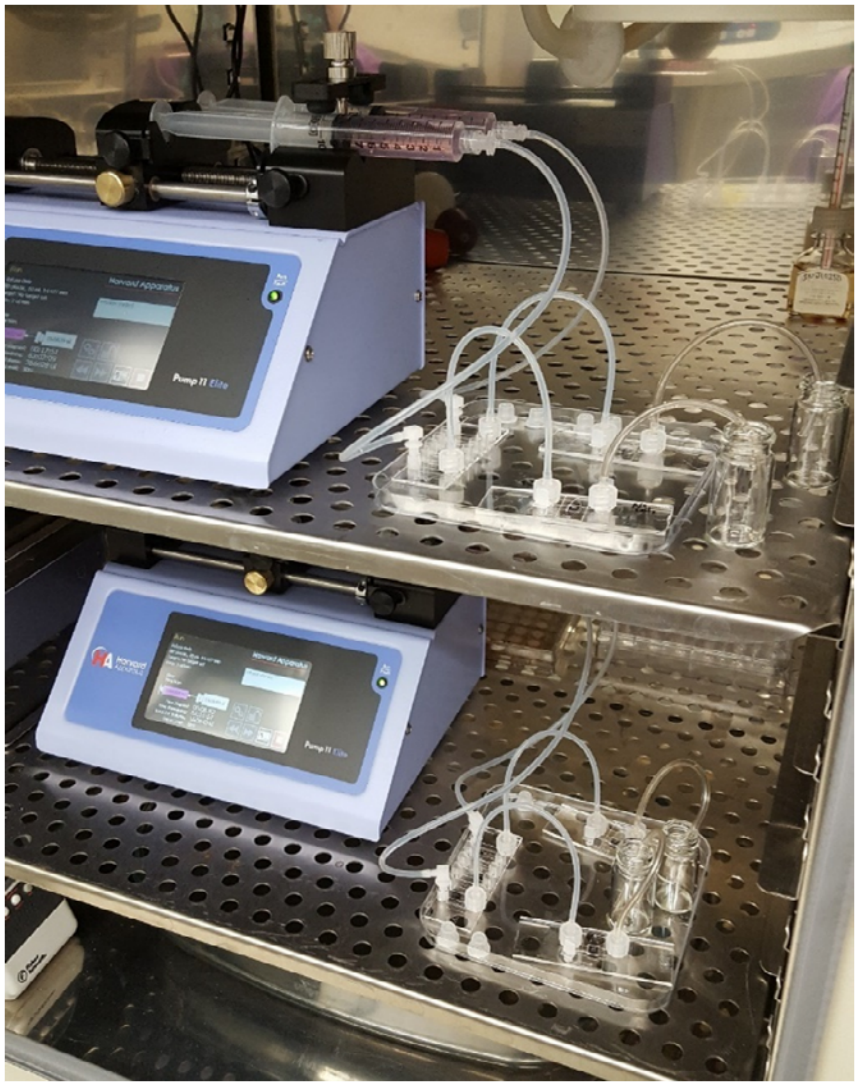
3D Dynamic

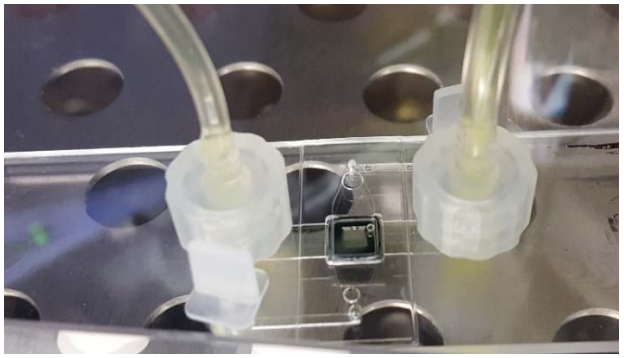


C. Experimental Timeline

Andrew Schwab et al., - Development of complementary 3-dimensional human neurovascular unit models using static transwells and dual-compartment microfluidic devices. In Revision

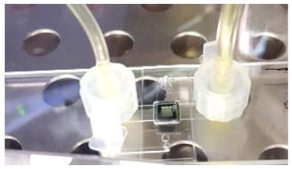
Current Model for Microphysiological Chips

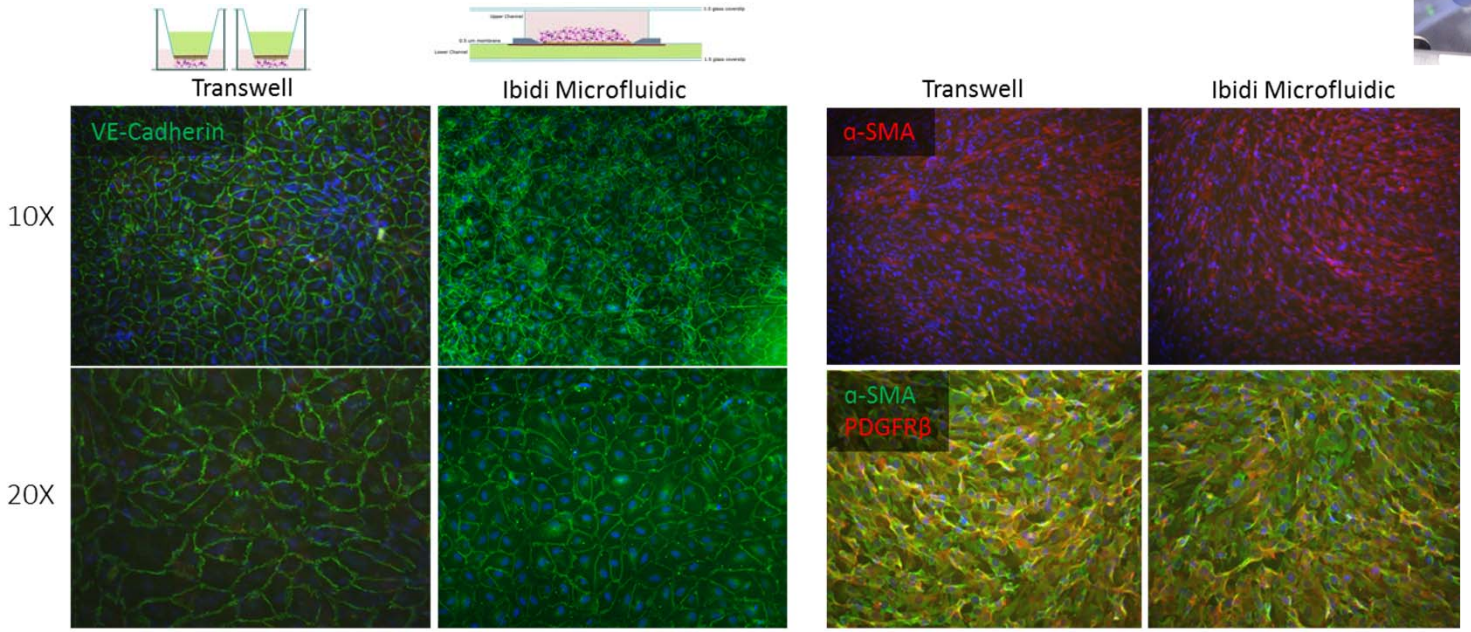


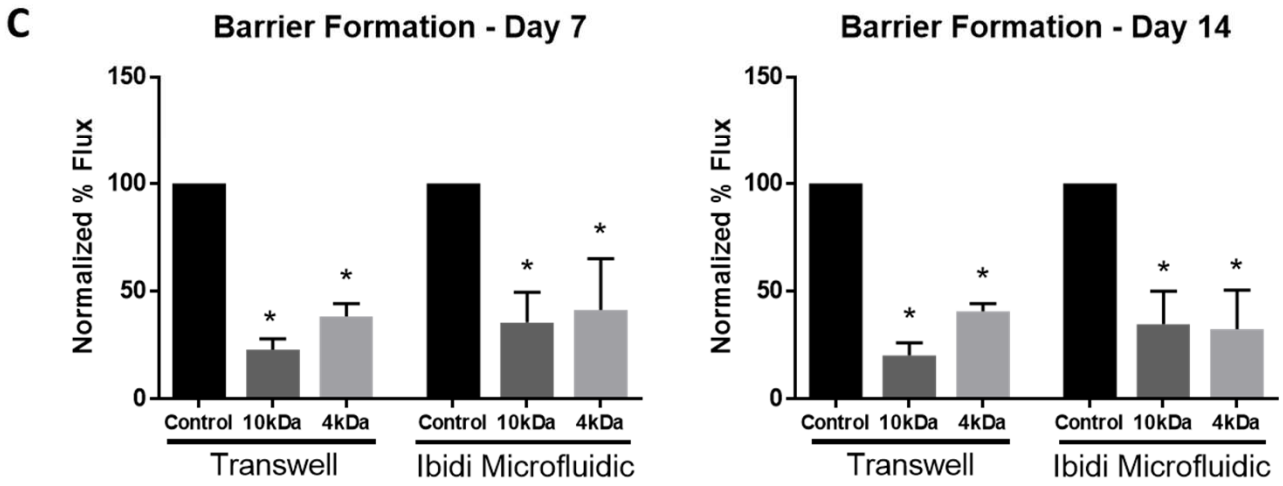


Single Pass media
2 µl per minute flow rate

Barrier Formation and Function in embryo-NVU

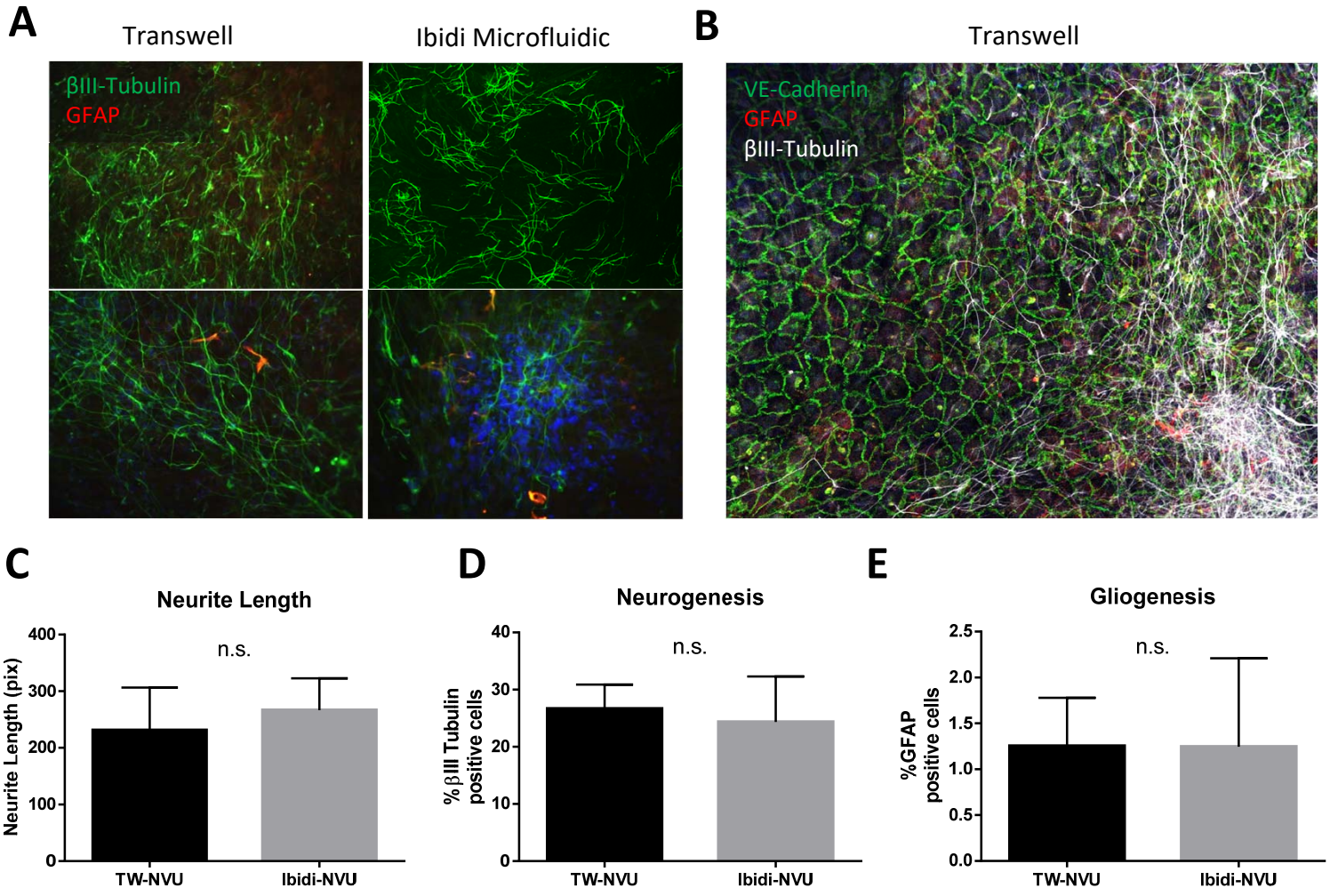






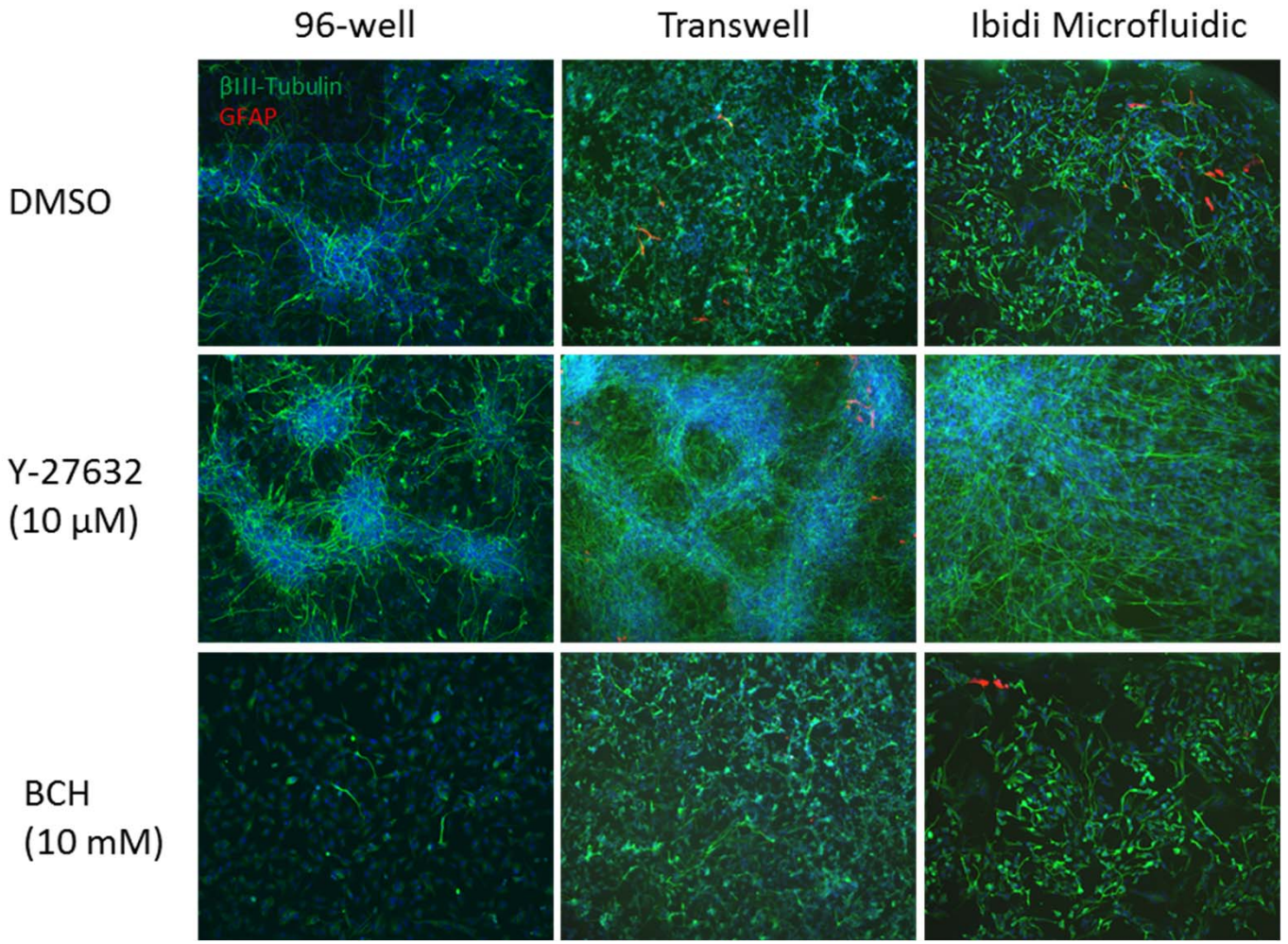
Andrew Schwab et al., - Development of complementary 3-dimensional human neurovascular unit models using static transwells and dual-compartment microfluidic devices. In Revision

Neural and Glial Differentiation are Robust in both Models



Andrew Schwab et al., - Development of complementary 3-dimensional human neurovascular unit models using static transwells and dual-compartment microfluidic devices. In Revision

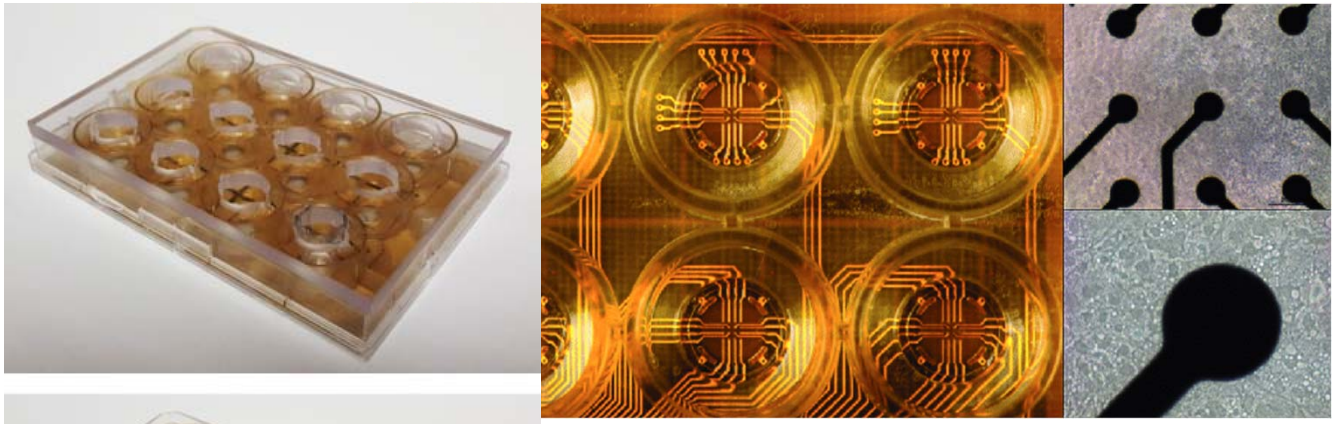
Chemical effects in the embryo-NVU
2D-3D: Neurons & Astrocytes

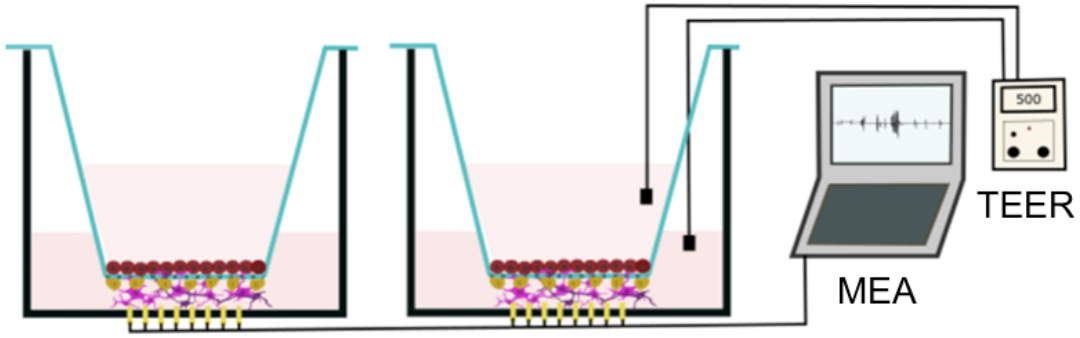


BCH = 2-amino-2-Norbornanecarboxylic acid

Andrew Schwab et al., - Development of complementary 3-dimensional human neurovascular unit models using static transwells and dual-compartment microfluidic devices. In Revision

Application of embryo-NVU Barrier to address issues of Developmental Neurotoxicity

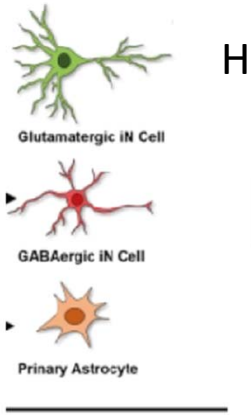




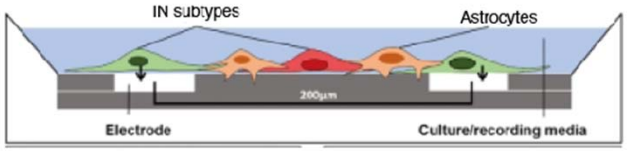
Human embryo early fetal blood-brain barrier transwell
Rat cortical neuron cultures

Collaboration with Dr. Tim Shafer's laboratory. Multi-Electrode Array analysis of chemicals

Direct Comparison of Human and Rat Neural Cultures to Chemical effects

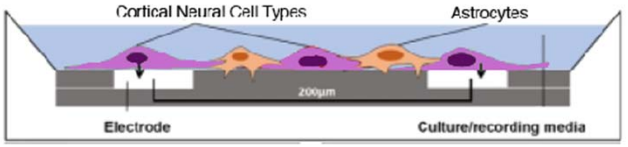


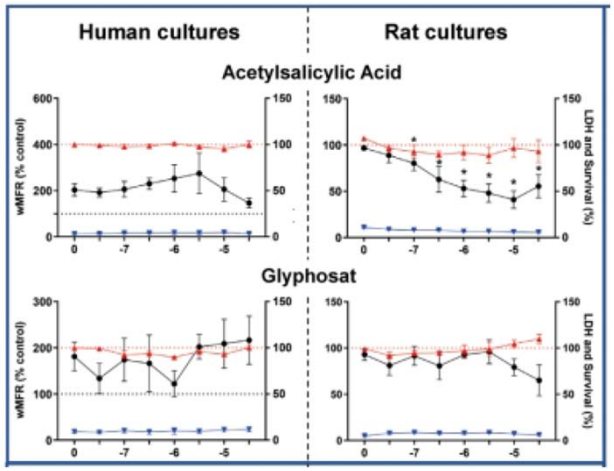
Human Model

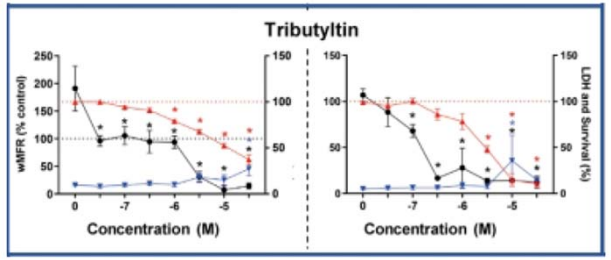


Rat Model

Rat Cortex



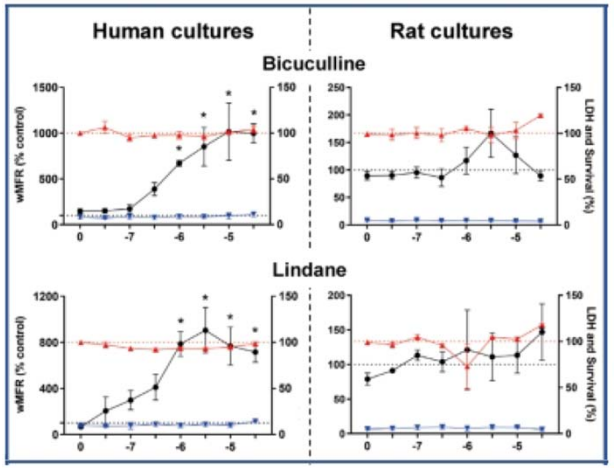




Cytotoxicity

Network Activity

LDH Release



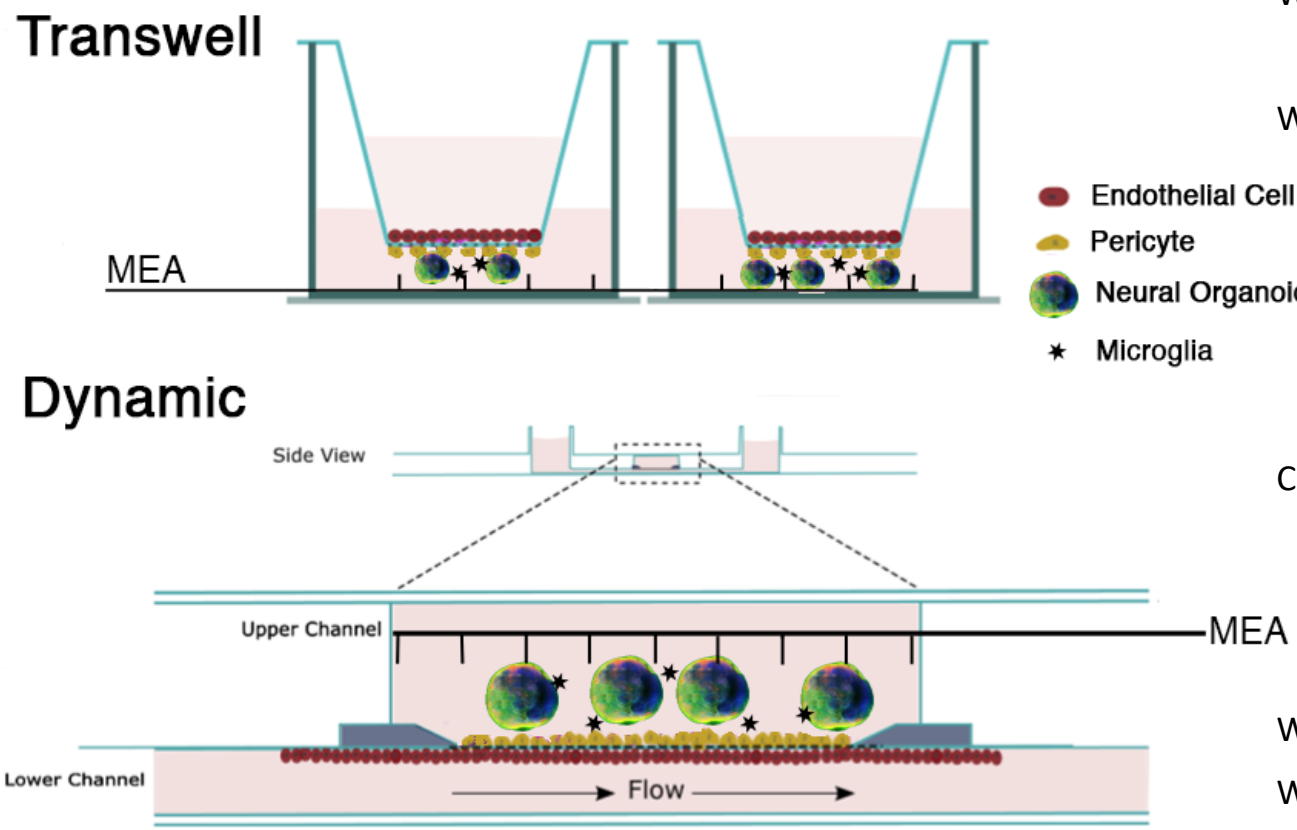
Comparison of Acute Effects of Neurotoxic Compounds on Network Activity in Human and Rodent Neural Cultures

L Saavedra[1,2]*, K Wallace[3]*, TF Freudenrich*[3], M Mall[2,4], WR Mundy[3], J Davila[1,2], TJ Shafer[3], M Wernig[2], and D Haag[1,2]

[1]NeuCyte Inc., San Carlos, CA, [2]Institute for Stem Cell Biology and Regenerative Medicine, Dept of Pathology, Stanford University School of Medicine, Stanford, CA, [3]BCTD, CCTE, ORD, US EPA, [4]Cell Fate Engineering and Disease Modeling Group, German Cancer research center (DKFZ) and DKFZ-ZMBH Alliance, Heidelberg, Germany. Tox Sci. In Press

*Authors contributed equally

Next Generation Gestational MEA-NVU – What are the critical pieces



What Species and Tissue –

Human

What Biological Process(es) –

Neuroprogenitor proliferation

Neural and Glia differentiation

Network attainment

Network activity

Cellular Complexity of Model

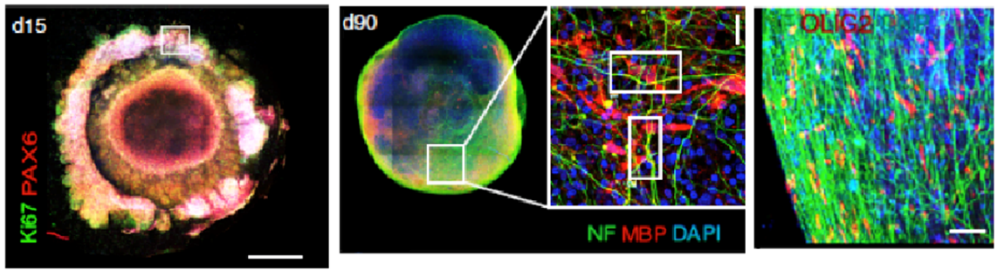
Neuroprogenitor Neural Organoid

Stem Cell-derived Model

Self-organization and assembly

Which Organoid Models

What qualifies cells for the model

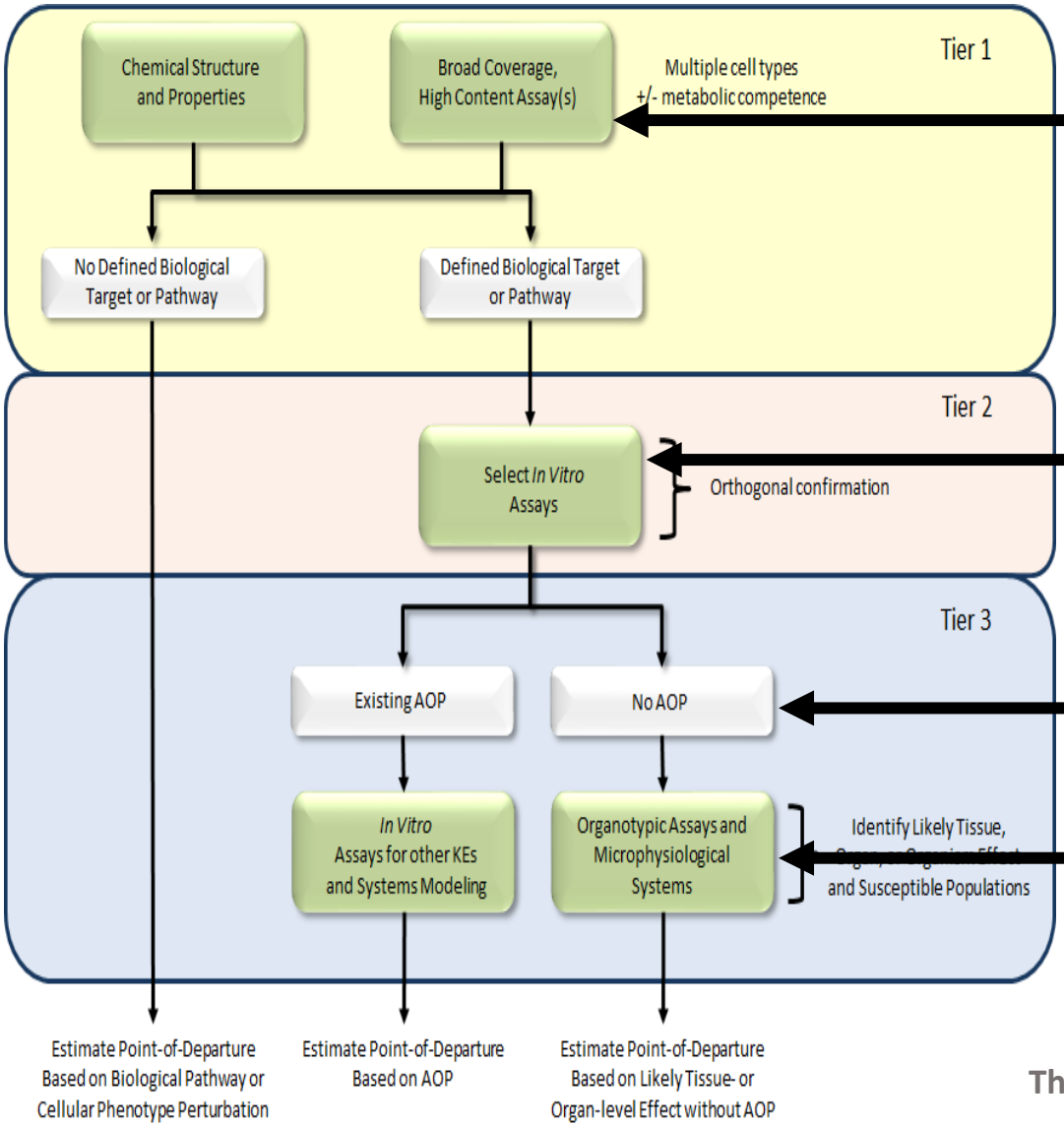


https://doi.org/10.1038/s41467-020-17521-w OPEN

Developmental GABA polarity switch and neuronal plasticity in Bioengineered Neuronal Organoids

Maria-Patapia Zafeiriou[1,2,3✉], Guobin Bao[1,2,4], James Hudson[1,2,5], Rashi Halder[6,7], Alica Blenkle[1], Marie-Kristin Schreiber[1], Andre Fischer[3,7,8], Detlev Schild[4] & Wolfram-Hubertus Zimmermann[1,2,3✉]

Tiered Hazard Evaluation Approach



2D neural cell toxicity
2D Cell painting in neuroprogenitor cells

Current 2D MEA Network Formation

AOPs for DNT

Embryo-NVU
Gestational MEA-NVU

The NexGen Blueprint of CompTox as USEPA
Tox. Sci. 2019; 169(2):317-322